Factors Influencing Cadmium Mobilization by Diethyldithiocarbamate: Chelator Dose, Cadmium Burden, and Sex*

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ABSTRACT

A study was made of the effects of diethyldithiocarbamate (DDTC) on organ distribution and excretion of cadmium (Cd) as influenced by DDTC dose, Cd burden, and sex of mice. One hundred to 250 mg per kg of DDTC did not promote mobilization of hepatic Cd, but it was effective at 500 mg per kg. All doses tested were significantly effective in mobilizing renal and splenic Cd, and the response was dose-dependent, as was the increase in brain levels of Cd. Cadmium mobilization by DDTC following Cd loads over a three-fold range appeared to be first order in nature; i.e., a given regimen of DDTC treatment promoted mobilization of a virtually constant percentage of the Cd administered, rather than a constant amount of Cd. The response to DDTC was markedly sex-dependent, being more effective in females than males. In organs of control Cd-loaded mice one sex-related difference was noted; spleens of females retained a considerably greater percentage of administered Cd than those of males.

Introduction

Diethyldithiocarbamate (DDTC) has been shown to be an effective antagonist of acute cadmium (Cd) toxicity in mice. While other chelators are most effective when given with or shortly after Cd, the antidotal effectiveness of DDTC was enhanced by delaying treatment for 30 min to four hours. A subsequent study showed that DDTC was effective in mobilizing and promoting fecal excretion of metallothioinein-bound Cd. Other investigators employed additional chelators as antagonists of acute Cd toxicity. Diethylenetriaminepentaacetate (DTPA) and dimercaptosuccinate (DMSA) in particular have been shown to confer considerable protection when given shortly after a >LD₁₀₀ dose of Cd. Cantilena and Klaassen found the relative efficacies to be of the order of...
DTPA > DMSA > DDTC. Similar results were obtained by Basinger et al. In contrast, when these three chelators were evaluated using multiple injections at equimolar doses following an injection of a non-lethal dose of Cd, DDTC was far superior to DTPA in promoting mobilization and excretion of Cd, and DMSA was without effect.

The purpose of the present study was to assess the influence of DDTC dose, cadmium burden, and sex on distribution, mobilization, and excretion of sequestered Cd.

Materials and Methods

Male and female mice of the (DBA/2 × C57BL/6)F₁ strain (BDF₁)* were used. Diethyldithiocarbamate (sodium salt, trihydrate)† was dissolved in 1.0 percent NaHCO₃ solution for i.p. injection. The CdCl₂ used was the hemipentahydrate‡, and the ¹⁰⁹CdCl₂ was carrier-free. $ For studies of DDTC dose, 80 male mice (24.7 ± 1.8 g) were injected i.p. with 0.03 mg of CdCl₂·2.5 H₂O containing 1.0 μCi of ¹⁰⁹Cd in 1.0 ml of 0.9 percent NaCl solution. The mice were maintained at 72 to 74°F, 40 to 60 percent relative humidity, with 12-hr light and dark cycles. Fifteen days later mice were divided into four equal groups. One group served as controls and received the appropriate volume of 1.0 percent NaHCO₃ solution at each injection. Each of the other three groups received 100, 250, or 500 mg per kg of DDTC, given as 1.0 ml per 30 g of body weight. Treatment was on a thrice weekly schedule. Two days after the seventh injection, six mice from each group were sacrificed by cervical dislocation, and organs removed for determination of radioactivity. The remaining mice received six additional injections of DDTC over the succeeding two-week interval, and organs were counted for radioactivity.

To assess the effect of Cd burden on efficacy of DDTC at a single dose level, 120 male mice (24.6 ± 1.9 g) were divided into six equal groups. Two groups received 0.03 mg, two received 0.06 mg, and two received 0.09 mg of CdCl₂·2.5 H₂O. In each injection, the volume given contained 1.0 μCi of ¹⁰⁹Cd. One group of 20 mice at each Cd dose level served as controls, while each of the other three groups received DDTC, 500 mg per kg. Measurements of whole-body radioactivity were performed one day after the seventh and 13th injection, and radioactive counts of organs were done. After 13 injections, the remaining mice were kept for an additional 23 days, following which organ radioactivity measurements were again performed.

The influence of sex on Cd distribution and pharmacologic action of DDTC was evaluated similarly. Twenty mice of each sex (males, 19.7 ± 0.5 g; females, 19.6 ± 0.4 g) were given 0.03 mg of CdCl₂·2.5 H₂O containing 1.0 μCi of ¹⁰⁹Cd and kept for 15 days. Treatment of 10 mice of each sex was then begun with DDTC, 500 mg per kg, on a thrice weekly schedule for a total of nine injections; 10 control mice of each sex received, each treatment day, an appropriate volume of 1.0 percent NaHCO₃ solution. One day after the third, sixth, and ninth injections, mice were subjected to whole-body gamma counting. On the following day, all mice were sacrificed for measurements of organ radioactivity.

Whole-body gamma counts and radioactivity in individual organs were measured as reported previously. All data were calculated as percent of the administered Cd dose remaining in the whole body or in individual organs by reference to the appropriate standards prepared on the same day as the Cd injections.

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Results

Influence of DDTC Dose

The Cd content of organs following seven injections of DDTC at 100, 250, and 500 mg per kg, expressed as percent of the administered Cd, are shown in figure 1. A biphasic effect with dose was noted in regard to response of liver Cd. At the two lower doses there was a small dose-related increase in liver Cd burden. At the highest dose there was a 17 percent reduction, accompanied by a reduction of whole-body burden of 24 percent. Thus, the relatively small reduction of hepatic Cd elicited by seven injections of DDTC contributed substantially to the reduction of total body burden. In contrast, there was a dose-dependent decrease in renal and splenic Cd levels. The large increase in brain Cd levels was also obviously dose-dependent.

Following 13 injections of DDTC, a similar distribution pattern was obtained as shown in figure 2. The elevations in liver counts were not statistically significant, but the reductions of hepatic, renal, and splenic burdens in those treated with the highest DDTC dose were marked. The sum of the percent reduction of total body Cd accounted for by these three or-

**Figure 1.** Retention of Cd in selected mouse organs following seven injections of DDTC at 0 (control), 100, 250, and 500 mg per kg per injection, expressed as percent of administered Cd. Data are presented as mean (n = 6) + or - 1.0 S.D. Organs assessed were liver (Li), kidney (Ki), spleen (Sp), and brain (Br). Levels of statistical differences were determined by analysis of variance (ANOVA) in comparison with each appropriate control Cd level.

**Figure 2.** Same as figure 1, except mice received a total of 13 injections of DDTC at designated doses.
gans was 29 percent of the administered dose, while whole-body gamma counts one day prior to sacrifice revealed a mean reduction of 35 percent. Therefore, 84 percent of the reduction of total body burden was the result of Cd depletion in these three organs, and the preponderance of Cd mobilized and excreted was from the liver (20 percent).

Influence of Cd Burden

The distribution patterns in control organs following three different Cd loading doses, along with the effects of seven injections of DDTC, 500 mg per kg, on these patterns, are shown in figure 3. In control liver, there was some reduction of percent retained Cd with increased Cd load, and the percent reduction of hepatic Cd burden was related to the Cd dose. In mice which received 0.03, 0.06, and 0.09 mg of CdCl₂·2.5 H₂O, the respective mean reductions following seven injections were 19, 25, and 27 percent, respectively. In kidney, the respective mean percent reductions were remarkably constant at 76 percent. A similar pattern was noted in spleen, with respective mean reductions of 70 to 75 percent. The calculated mean values in brain Cd levels appeared to increase (611, 711, and 1017 percent, respectively).

After 13 DDTC injections, the reductions in liver, kidney, and spleen followed a similar pattern but were more pronounced (figure 4). With an increasing Cd burden, the mean reductions in he-

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**Figure 3.** Retention of Cd in selected organs from mice which received various Cd loads prior to treatment with seven injections of DDTC at 500 mg per kg. All other conditions were as described for figure 1.

**Figure 4.** Same as figure 3, except mice received a total of 13 injections of DDTC, 500 mg per kg.
patic Cd were 34 to 43 percent. In kidney, the mean reductions were 83 to 86 percent, while the corresponding splenic reductions were also relatively constant at 76 to 79 percent. The DDTC-induced elevations in brain Cd levels again appeared to increase with increased Cd burden (575, 663, and 736 percent, respectively).

The data on whole-body gamma counts, performed one day prior to sacrifice of mice, are shown in figure 5, and are altogether consistent with the data obtained from individual organ counts (figure 4). At Cd loads of 0.03, 0.06, and 0.09 mg, the control mice retained 90, 80, and 75 percent of the injected doses, corresponding to respective retentions of 0.027, 0.048, and 0.067 mg in terms of CdCl$_2$·2.5 H$_2$O. However, the respective mean reductions were relatively consistent at 33 to 39 percent with increasing Cd burden.

The remaining mice which received increasing amounts of Cd followed by 13 injections of DDTC were kept for 23 days, after which six from each group were sacrificed and organ radioactivity was assessed. There was only slight redistribution of Cd during the period in which treatment was withheld (figure 6). There were only minor reductions of Cd levels in livers of control mice, accompanied by modest increases in control kidney burdens. Irrespective of the level of original Cd burden, there was no major redistribution of Cd from livers to kidneys of treated mice.

**Influence of Sex**

The male and female mice, closely matched by weight (intergroup and intragroup) received a total of nine injections of DDTC, 500 mg per kg, on a thrice weekly basis; treatment was begun 15 days after all mice were given 0.03 mg of CdCl$_2$·2.5 H$_2$O along with 1.0 $\mu$Ci of $^{109}$Cd. One day after the third, sixth, and ninth injection, mice were subjected to whole-body gamma counting. Results are shown in figure 7. Least squares regression analysis suggested a modest increase in percent of administered Cd in both male and female controls, but this was most likely due to the rather large standard deviations of the means. There was a more rapid depletion of Cd from females than from males. The mean percent reduction (compared to control values) in males after nine injections was 18 percent, while in females it was 35 percent ($p = <0.001$).

On the second day after the ninth injection, all mice were sacrificed and radioactivity of organs was measured. Results are shown in figure 8 and are virtually identical to the data obtained in a subsequent duplicate procedure. Of
those organs for which direct comparisons could be made between males and females, control values were substantially identical in both sexes \((p > 0.05)\) with the singular exception of spleen; spleens from control female mice retained about 125 percent more of the administered Cd than the spleens of the control males \((0.56 \pm 0.11 \text{ percent compared with } 0.25 \pm 0.05 \text{ percent}; p < 0.001)\). As there were no significant differences between the sexes regarding splenic weights \((\text{males, } 100 \pm 21 \text{ mg}; \text{females, } 96 \pm 13 \text{ mg}; p = 0.664)\), body weights \((\text{males, } 23.2 \pm 2.8 \text{ g}; \text{females, } 22.1 \pm 0.7 \text{ g}; p = 0.30)\), or spleen weight/body weight \((\times 100)\) ratios \((\text{males, } 0.43 \pm 0.09; \text{females, } 0.43 \pm 0.05; p = 0.96)\), this indicates a relatively greater affinity of splenic tissue of females for Cd than was observed in males.

Regarding influence of sex on response of specific organs to treatment, the trend was similar for both sexes, but some definite quantitative differences were discerned. Perhaps the most significant of these was the effect on hepatic Cd. In males, treatment with DDTC reduced the percent of administered Cd from a mean control value of 57 percent to 40 percent. In females, the mean control value of 58 percent was reduced to 31 percent \((p < 0.001)\). In contrast, the percent reduction of renal Cd by DDTC in females was slightly but significantly \((p < 0.001)\) less than in males. The previously-noted increase in testicular Cd evoked by DDTC in male mice was again observed, while ovarian levels in females were reduced markedly.

**Discussion**

In the first part of the present study, it became quite evident that reduction by DDTC of Cd burden from its major sites
of sequestration is dose-dependent. Indeed, following seven injections, response of liver Cd was biphasic; there were slight but significant elevations of hepatic Cd burden at the two lower doses, with reduction occurring only at the highest DDTC dose. However, there were consistent reductions of renal and splenic Cd burdens at all DDTC doses, accompanied by dose-dependent increases in brain levels.

In contrast to DDTC dose dependency, the percent reductions by DDTC of Cd levels in kidney and spleen upon administration of fixed doses of DDTC were remarkably constant despite increasing Cd burdens. While it was reported earlier that excretion of Cd by control and DDTC-treated mice appeared to follow a zero order kinetic pattern,6,7 the present observations of organ depletion of a virtually constant percentage of Cd burden by a given DDTC dose regimen are strongly suggestive of a pattern of organ mobilization which follows first order kinetics. If this occurs in man, it could have considerable clinical implications for those with severe chronic Cd intoxication.

Regarding influence of sex on Cd toxicity, distribution, or mobilization, observations of human cases of Itai-itai disease in the Toyama Prefecture in Japan revealed that most of the patients were multiparous (mean = 6), postmenopausal women; one tabulation listed 297 females and 122 males, and another listed 184 females and only 33 males.8 In animal studies of differential responses to Cd as related to sex, Johns et al reported that male rats receiving a diet containing CdCl₂ at 125 ppm all died within 50 days, while most female rats on the same diet survived much longer.9 More recent animal studies have focused primarily on the effect of Cd on liver microsomal enzyme systems as influenced by sex. Hadley et al found that Cd potentiates hexobarbital-induced sleep times in male but not in female rats; however, when Swiss albino mice were used, no differences were observed.9 Similar observations with rats were made by Schnell et al, who attempted to link the sex-related differences to Cd-induced testicular necrosis. The decreased testosterone production could indirectly result in decreased hepatic drug metabolism.10 However, in a subsequent study, it was shown that while the rate of hexobarbital metabolism was much higher in hepatic microsomal fractions from male than female rats, the former were much more sensitive to Cd inhibition in vitro than were the latter.11 Ando confirmed the extreme Cd sensitivity of drug-metabolizing enzyme systems in male rats, and proposed that the mechanism was partially due to the conversion of Cd of cytochrome P-450 to cytochrome P-420.1
None of the animal studies cited previously\(^1,9,10,11,12\) dealt with effects of Cd antagonists and thus failed to elicit any obvious link between sex of the test animal and the rather striking sex-dependent differences in response to DDTC treatment noted in the present study. In addition, the enhanced splenic levels of Cd found in control females compared to control males in this study was unexpected, as the spleen is not generally considered to be influenced by sex hormones. As the observation of enhanced responsiveness of Cd-laden female mice to the salutary effect of DDTC may have some practical implications, this clearly seems worthy of additional study.

References